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Genetic Susceptibility to Distinct Bladder Cancer Subphenotypes

Lin T. Guey[a,e], **Montserrat García-Closas**[b], **Cristiane Murta-Nascimento**[e], **Josep Lloreta**[c,d], **Laia Palencia**[e], **Manolis Kogevinas**[e,f,g,h], **Nathaniel Rothman**[b], **Gemma Vellalta**[e], **Malu Calle**[i], **Gaëlle Marenne**[a], **Adonina Tardón**[j], **Alfredo Carrato**[k], **Reina García-Closas**[l], **Consol Serra**[m], **Debra T Silverman**[b], **Stephen Chanock**[b], **Francisco X. Real**[a,d,e], and **Núria Malats**[a,e,*] **for the EPICURO/Spanish Bladder Cancer Study investigators**[n]

[a]Spanish National Cancer Research Centre (CNIO), Madrid, Spain

[b]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Department of Health and Human Services, Bethesda, MD, USA

[c]Departament de Patologia, Hospital del Mar – IMAS, Barcelona, Spain

[d]Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain

[e]Institut Municipal d'Investigació Mèdica – Hospital del Mar, Barcelona, Spain

[f]Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain

[g]University of Crete, Heraklion, Greece

[h]CIBER en Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

[i]University of Vic, Vic, Spain

[j]Universidad de Oviedo, Oviedo, Spain

[*]**Corresponding author** Spanish National Cancer Research Centre (CNIO), Melchor Fernández Almagro, 3, E-28029, Madrid, Spain, nuria@cnio.es.
[n]EPICURO/Spanish Bladder Cancer Study Investigators in Annex 1

Author contributions: Núria Malats had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Malats, Real, Kogevinas, Rothman, Silverman, Dosemeci, García-Closas, Murta-Nascimento, Chanock.
Acquisition of data: Malats, Real, Kogevinas, Rothman, Silverman, Dosemeci, García-Closas, Tardón, Serra, Carrato, García-Closas, Murta-Nascimento, Chanock, Lloreta.
Analysis and interpretation of data: Guey, Malats, Real, Kogevinas, Rothman, Silverman, Dosemeci, García-Closas.
Drafting of the manuscript: Guey, Malats, Real.
Critical revision of the manuscript for important intellectual content: Guey, Malats, Real, Kogevinas, Rothman, Silverman, Dosemeci, García-Closas, Tardón, Serra, Carrato, García-Closas, Murta-Nascimento, Chanock, Calle.
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[k]Hospital Universitario de Elche, Elche, Spain

[l]Unidad de Investigación, Hospital Universitario de Canarias, La Laguna, Spain

[m]Consorci Hospitalari Parc Taulí, Sabadell, Spain

Abstract

Background—Clinical, pathologic, and molecular evidence indicate that bladder cancer is heterogeneous with pathologic/molecular features that define distinct subphenotypes with different prognoses. It is conceivable that specific patterns of genetic susceptibility are associated with particular subphenotypes.

Objective—To examine evidence for the contribution of germline genetic variation to bladder cancer heterogeneity.

Design, setting, and participants—The Spanish Bladder Cancer/EPICURO Study is a case-control study based in 18 hospitals located in five areas in Spain. Cases were patients with a newly diagnosed, histologically confirmed, urothelial cell carcinoma of the bladder from 1998 to 2001. Case diagnoses were reviewed and uniformly classified by pathologists following the World Health Organisation/International Society of Urological Pathology 1999 criteria. Controls were hospital-matched patients (n = 1149).

Measurements—A total of 1526 candidate variants in 423 candidate genes were analysed. Three distinct subphenotypes were defined according to stage and grade: low-grade nonmuscle invasive (n = 586), high-grade nonmuscle invasive (n = 219), and muscle invasive (n = 246). The association between each variant and subphenotype was assessed by polytomous risk models adjusting for potential confounders. Heterogeneity in genetic susceptibility among subphenotypes was also tested.

Results and limitations—Two established bladder cancer susceptibility genotypes, *NAT2* slow-acetylation and *GSTM1*-null, exhibited similar associations among the subphenotypes, as did *VEGF*-rs25648, which was previously identified in our study. Other variants conferred risks for specific tumour subphenotypes such as *PMS2*-rs6463524 and *CD4*-rs3213427 (respective heterogeneity p values of 0.006 and 0.004), which were associated with muscle-invasive tumours (per-allele odds ratios [95% confidence interval] of 0.56 [0.41–0.77] and 0.71 [0.57–0.88], respectively) but not with non–muscle-invasive tumours. Heterogeneity p values were not robust in multiple testing according to their false-discovery rate.

Conclusions—These exploratory analyses suggest that genetic susceptibility loci might be related to the molecular/pathologic diversity of bladder cancer. Validation through large-scale replication studies and the study of additional genes and single nucleotide polymorphisms are required.

Keywords

Urinary bladder cancer; Genetic polymorphism; Heterogeneity; Tumour subphenotypes; Pathologic characteristics

1. Introduction

Urothelial cell carcinoma of the bladder (UCCB) is the fifth most common cancer in men; it occurs with a male-to-female ratio of approximately 3:1. Spain has one of the highest incidence rates among men (55 per 100 000) yet one of the lowest among women (7.4 per 100 000), with a male-to-female ratio of 7:1 [1]. Because of its commonly indolent course, UCCB is one of the most expensive cancers in terms of medical care costs per patient [2].

Pathologic and molecular evidence suggest that UCCB is not a unique phenotype. Based on stage, tumours are classified as non–muscle invasive and muscle invasive, the former representing approximately 80% of cases at presentation. Non–muscle-invasive tumours are subclassified into low and high grade. Tumours of these subphenotypes have different biologic behaviour and are associated with distinct patient prognosis [3–5]. This classification has been supported by the identification of molecular alterations that identify two major genetic pathways leading to low-grade, genomic stable and high-grade, genomic unstable tumours, the latter including a proportion of non–muscle-invasive tumours and the vast majority of muscle-invasive tumours. Activating mutations in *FGFR3* and *PI3KCA* are associated with low-grade non–muscle-invasive tumours, whereas p53 and RB pathway alterations are critical for high-grade nonmuscle-invasive and muscle-invasive tumours (Fig. 1) [6,7]. However, the precise mechanisms through which tumours evolve need to be more precisely defined.

The aetiology of UCCB is multifactorial, with both environmental and genetic factors identified. The best established exogenous risk factors are tobacco and occupational exposure to aniline dyes and aromatic amines [8,9]. Their influence on UCCB risk is similar for tumours of varying stages and grades [10]; thus exogenous risk factors cannot currently explain disease heterogeneity. UCCB is also one of the tumours for which a role of low-penetrance genetic variants, such as *GSTM1*-null and *NAT2*-slow, has been best established [11]. Furthermore, strong evidence indicates an interaction between the *NAT2*-slow variant and smoking [11]. Other genetic polymorphisms have been reported to be associated with UCCB risk, but replication is necessary to establish risk conclusively [12,13]. One explanation for this lack of success is that polymorphisms may exhibit a small to moderate effect only within particular subphenotypes of the disease [6,14]. Thus polymorphisms that may be important for a particular subphenotype could be overlooked in an analysis that pools the subphenotypes due to an attenuation of effects. The aim of this work is to investigate whether UCCB subphenotypes defined according to clinicopathologic characteristics have different associations with germline polymorphisms in the Spanish Bladder Cancer (SBC)/EPICURO Study. Although this approach has been recently applied to breast [14] and prostate cancer [15], this report is the first to do so for UCCB. A total of 1526 polymorphisms in 423 cancer candidate genes were examined with respect to three distinct UCCB subphenotype groups.

2. Patients and methods

2.1. Study population

The details of the study population have been described previously [8,11,16]. Briefly, the SBC/EPICURO Study is a case-control study based in 18 hospitals located in five areas in Spain. Cases were patients with a newly diagnosed, histologically confirmed UCCB from 1998 to 2001. A panel of expert pathologists reviewed slides to confirm the diagnosis and ensure uniformity of the classification criteria according to the 1998 system of the World Health Organisation and the International Society of Urological Pathology [3]. The TNM classification was applied for stage assignment [17]. Controls were patients admitted to participating hospitals for diagnoses believed to be unrelated to the exposures of interest and were individually matched to cases on age, gender, ethnic origin, and region. Information was obtained during the first hospital admission. A total of 1219 cases and 1271 controls agreed to participate in the study and were interviewed. Of them, 1188 cases (97%) and 1173 controls (92%) provided a blood or buccal cell sample. Exclusions were due to insufficient DNA and to reduce heterogeneity (nonwhite, lack of diagnostic slides, nontransitional histology, and urothelial neoplasms of low malignant potential). The final study population consisted of 1051 cases and 1149 controls. Subsequently, cases were classified in three subphenotypes according to tumour stage (T) and histologic grade (G):

low-grade non–muscle invasive (Ta G1/Ta G2, $n = 586$), high-grade non–muscle invasive (Ta G3/T1 G2/T1 G3, $n = 219$), and muscle invasive (T≥2, $n = 246$).

2.2. Genotyping

Genotyping was performed as described using leukocyte (1011 cases, 1032 controls) or mouthwash (40 cases, 117 controls) DNA and the GoldenGate (Illumina, San Diego, CA, USA) and/or TaqMan (Applied Biosystems) assays [11,12]. The GoldenGate assay was comprised of single nucleotide polymorphisms (SNPs) from the SNP500Cancer project (http://snp500cancer.nci.nih.gov) belonging to candidate genes related to the carcinogenic process; an attempt was made to select for nonsynonymous variants or those with evidence for functional significance [12]. All polymorphisms were SNPs, with the exception of *NAT1*, which was categorised into *NAT1**4 and *NAT1**10, the "at-risk" allele [11]; *NAT2*, which was categorised into slow-acetylator and intermediate/rapid-acetylator phenotypes; and *GSTM1* and *GSTT1* genotypes, which were defined as "null" (−/−) and "present" (+/− or +/+) [11]. SNPs are reported as the gene name followed by the reference SNP (rs) number, according to the international coding nomenclature. Details regarding all 1526 polymorphisms can be found in Supplementary Table 1.

Genotype concordance in the duplicate quality-control samples was ≥99%. For the 63 SNPs genotyped by both platforms, the concordance rate was ≥98%. For those SNPs, information from the Illumina platform was first applied, followed by TaqMan data in the event of missing information. Approximately 6% (96 of 1526) of the gene variants showed significant departures from Hardy-Weinberg equilibrium ($p < 0.05$) in the control population, consistent with what would be expected by chance (Supplementary Table 1). Polymorphisms were excluded if they had a low minor allele frequency (<0.05) in the control population or if they were highly correlated with other variants ($r^2 \geq 0.85$). A total of 451 variants were excluded (Supplementary Table 1).

2.3. Statistical methods

Demographic characteristics were compared among individuals in the three UCCB subphenotypes and in the control group using the analysis of variance for continuous variables and the χ^2 test of independence for categorical variables. Additionally, these comparisons were made among the three subphenotypes only.

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for the three UCCB subphenotypes using polytomous logistic regression models for each variant considering a codominant and additive mode of inheritance. The codominant model makes no assumptions regarding the contribution of the alleles in the three genotypes, whereas the additive model assumes a dose response or trend with every increase in allele count (0, 1, 2). The model compared each tumour group to the control group, adjusting for the following potential confounders: age at interview, gender, region and smoking status (never, occasional, former, and current) [8]. Thus an OR >1 indicates an increased risk and an OR <1 indicates a decreased risk for a particular subphenotype compared with the controls. The global association between variants and risk of any UCCB was tested using a likelihood ratio test (LRT) comparing models with and without all variant coefficients for both modes of inheritance. Similarly, the association between variants and risk of distinct subphenotypes was tested with a 2-df LRT and a per-allele trend test for each subphenotype.

Heterogeneity of genotype ORs (risk estimates) was tested using a LRT comparing a model in which the variant coefficients were constrained to be equal across the subphenotypes with an unconstrained model for both modes of inheritance. These LRTs are analogous to homogeneity of OR tests. Post hoc pairwise comparisons were performed if the

heterogeneity *p* value was <0.01. The *p* values <0.01 were considered statistically significant. The robustness of the results for nonredundant and common polymorphisms was evaluated using the false discovery rate (FDR) [18]. Rather than applying an arbitrary FDR cut-off threshold, the most significant findings are presented so that the reader may assess the robustness of the results. All analyses were performed in R v.2.6.0 (http://www.r-project.org).

3. Results

Patients with tumours of the three subphenotypes were similar with respect to all demographic characteristics except for age (Table 1). Cases with low-grade non–muscle-invasive tumours were slightly younger than those with high-grade non–muscle-invasive (mean age: 65.2 vs 67.3 yr) and muscle-invasive (mean age: 67.1 yr) tumours. Most patients were men (approximately 87%) and either former smokers (39.2%) or current smokers (43.0%). Patients with muscle-invasive tumours were more likely to be current smokers (47.2%) than those with low-grade (42.3%) and high-grade (40.2%) non–muscle-invasive tumours, although not significantly so ($p = 0.7$).

Controls were similar to cases with respect to gender and region, as expected. They were slightly younger (mean age: 64.7 yr) than cases in the high-grade non–muscle-invasive and muscle-invasive groups, although, again, these differences were quite small and there was no overall age difference between pooled cases and controls. As expected, controls were less likely to be current smokers than cases.

3.1. Heterogeneity in genetic susceptibility according to tumour subphenotypes

There was evidence of heterogeneity in genetic susceptibility ($p < 0.01$) to UCCB according to subphenotypes in 13 SNPs belonging to 12 genes under the codominant or the additive mode of inheritance (Table 2a and 2b, respectively). Four of them (*PMS2*-rs6463524, *DNAJC18*-rs4315920, *BRCA2*-rs1801406, and *XRCC5*-rs828702) showed significant heterogeneity *p* values under both modes of inheritance. *PMS2*-rs6463524 and *DNAJC18*-rs4315920 were only associated with the muscle-invasive group and provided the strongest evidence of heterogeneity in the genotype ORs under the codominant (respective *p* values, 0.002 and 0.005) and additive (respective *p* values, 0.006 and 0.001) modes of inheritance. These findings were not robust to multiple testing because all FDR values for the heterogeneity *p* values, based on the 1075 polymorphisms examined in the analyses, were >0.75.

Pairwise comparisons of the SNPs with significantly different risk estimates showed that the more extreme subphenotypes (low-grade non–muscle-invasive vs muscle-invasive) were significantly different ($p < 0.01$) under the additive model. This was not the case for the codominant model, where only three of the eight SNPs showed different risk estimates between the extreme groups. Pairwise comparisons also showed that risk estimates differed between the high-grade non–muscle-invasive and muscle-invasive groups under both modes of inheritance. In contrast, risk estimates between the low-grade and high-grade non–muscle-invasive groups were similar for all of these SNPs except for two (*MBL2*-rs5030737 and *MMP1*-rs10488) under the codominant model.

Interestingly, these 13 SNPs were all nonsignificant ($p > 0.01$) in a logistic regression analysis when patients in the three subphenotype groups were pooled. This is due to an attenuation of the risk estimates (Table 3), although the trend tests for the *MMP1*-rs10488 and *PMS2*-rs6463524 SNPs were marginally significant ($p = 0.04$).

Figure 2 displays the risk estimates and 95% CIs for the three subphenotypes separately and jointly under the additive pattern of inheritance for the following four SNPs: *CD4*-rs3213427, *PMS2*-rs6463524, *MMP1*-rs10488, and *ALAD*-rs1139488. These SNPs conferred increased (or decreased) risks for a particular subphenotype and neutral risks for the remaining tumour groups. Estimates for the *CD4*-rs3213427 and *PMS2*-rs6463524 SNPs were only significant for the muscle-invasive group, whereas estimates for the *MMP1*-rs10488 and *ALAD*-rs1139488 SNPs were significant only for the high-grade and low-grade non–muscle-invasive groups, respectively. The figure also shows the attenuation in risk estimates for the pooled tumour groups. Supplementary Table 2 displays all genetic association results for the three subphenotypes, including the genotype frequencies for each polymorphism.

3.2. Genetic variants associated with bladder cancer: GSTM1, nAT2

GSTM1-null and *NAT2*-slow genotypes provide the best evidence of genetic susceptibility to UCCB [11] and conferred similar risks among the three subphenotypes (Fig. 3a). The *GSTM1*-null genotype was associated ($p < 0.001$) with all three subphenotypes. The *NAT2*-slow genotype was associated with the non–muscle-invasive groups with p values < 0.009 and with the muscle-invasive group with $p = 0.05$.

3.3. Previously identified variants in the Spanish Bladder Cancer Study

Our previous reports suggested novel SNPs to be associated with UCCB risk [12]. We analysed their association with the tumour subphenotypes and focused on SNPs from the 13 top candidates reported (Table 1 of Garcia-Closas et al [12]). The most robust association with overall UCCB was for *VEGF*-rs25648 [12]; this variant was associated with all three subphenotypes: tumour-specific 2-df LRT $p < 0.008$ (Fig. 3b and Supplementary Table 3). The remaining SNPs in genes previously reported to be associated with UCCB also showed similar risks among the three subphenotypes ($p > 0.01$), but for the most part they were significantly associated with only one or two of the tumour groups because of a statistical power issue (Supplementary Table 3).

4. Discussion

We investigated evidence of heterogeneity of genetic susceptibility to three well-established UCCB subphenotypes with different biologic behaviours and prognoses, leading to the identification of novel tumour subgroup-specific associations. These findings require replication in future studies. Our aim was to identify gene variants that differentially predispose bladder tumours to papillary low-grade, genomically stable (α), high-grade non–muscle-invasive (β_1), or muscle-invasive (β_2) subphenotypes (Fig. 1). We previously found that an inherited *FGFR3* variant was differentially associated with low-grade versus high-grade non–muscle-invasive tumours [16].

Risk estimates for the two best established UCCB susceptibility genes, *GSTM1* and *NAT2*, were similar across subphenotypes. Similarly, *VEGF*-rs25648 and other polymorphisms previously identified to be associated with UCCB risk in our study [12] conferred similar risks among subphenotypes.

Among the patients with non–muscle-invasive tumours, risk estimates were similar for the SNPs analysed, except for *MBL2*-rs5030737 and *MMP1*-rs10488, which conferred significantly decreased risk for heterozygotes in high-grade tumours. Mannose-binding lectin 2 (*MBL2*) participates in innate immunity and rs5030737 (*R52C*) is associated with impaired function [20]. Other *MBL2* variants have been associated with risk of autoimmune and infectious diseases [20] and cancer [21,22]. Recently, Pine et al reported the prognostic

value of *MBL2* polymorphisms, including rs5030737, for lung cancer survival [23]. *MMP1* is involved in tissue remodelling, tumour invasion, and metastasis. Although associations found only for the heterozygous genotype are often disregarded because they are believed to be biologically implausible, there is precedent that some of them are detected consistently across studies [24,25]. Also, it is likely that these SNPs follow other modes of inheritance (eg, dominant or recessive) but, due to the exploratory nature of these analyses, only the codominant and additive modes of inheritance were considered. Another caveat is that, when stratified by subphenotypes, the genotype frequencies can be considerably low and thus add noise to the OR estimates (Supplementary Table 2c).

The most notable differences in risk estimates occurred when comparing muscle-invasive and non–muscle-invasive tumours. Irrespective of the mode of inheritance, heterogeneity was observed for *DNAJC18*-rs4315920, coding for a heat shock protein with methyl-transferase activity and SNPs in DNA repair genes, *PMS2*-rs6463524, *BRCA2*-rs1801406, and *XRCC5*-rs828702. There is some evidence on the role of genetic variation in double-strand break DNA repair mechanisms in UCCB susceptibility [13].

CD4-rs3213427 and *PMS2*-rs6463524 showed neutral risk estimates (OR: ≈1) for the non–muscle-invasive groups and decreased risk for the muscle-invasive group (Fig. 2 and Table 2). Other SNPs conferred risk estimates among subphenotypes in opposite directions. Although we cannot discard the possibility that this may be due to random variation or chance, it is possible that variants confer contrasting risks to different subgroups due to the complex mechanisms involved in the development/progression of tumours. Because the effect of each genetic variant is probably modulated by other genetic factors, both inherited and somatic, as well as by micro- and macroenvironmental exposures, such contrasting risks remain biologically plausible. Specific somatic alterations are involved in the different subphenotypes, possibly leading to distinct molecular profiles that may explain inherited factors in opposite directions. In fact, subgroup analyses in other studies have observed such crossover interactions [26,27], with some underlying debate about their biologic plausibility [28].

An additional aspect regarding the interpretation of the results relates to the functionality of the variants differently associated with bladder cancer subphenotypes. Some of them are placed in coding regions leading to a change of amino acid and for whom an altered protein function has been proved (23). Other significant SNPs, although placed in coding regions, do not lead to an amino acid change. This fact does not rule out a functional effect because it has been reported that SNPs can be an important mechanism affecting splicing regulation (29,30). Furthermore, it is noteworthy that most of the very reproducible “hits” in genome-wide association studies identified over the past 2–3 yr indeed are placed in gene deserts or in areas of unknown function; the findings remain highly relevant (A Catalog of Published Genome-Wide Association Studies; available at: http://www.genome.gov/26525384). Among them, that reported by Kiemeney et al was highly associated with bladder cancer risk [31].

The study’s strengths include a large sample size, high participation rates, high quality of information on exposures and genotyping, and the uniform pathologic assessment of tumours. Furthermore, this study was prospectively designed to assess the role of genetic, environmental, and clinical factors in UCCB risk. Nonetheless, the results reported here should be interpreted with caution in light of several limitations. The genes/SNPs analysed were not selected to assess subphenotype heterogeneity. Some subjectivity in the pathologic evaluation of tumours cannot be ruled out, although it was reduced by ensuring a uniform tumour classification by trained pathologists. By considering molecular alterations occurring early in urothelial carcinogenesis, a more accurate subphenotyping classification may be

applied in future studies. Furthermore, the study was based on a tumour development/progression model that is likely to be an oversimplification. Although rare, a few patients with low-grade tumours eventually invade the muscle; furthermore, confluence of tumours can also occur in the context of a multicentric neoplasm such as UCCB. Although we observed some evidence of heterogeneity in genetic susceptibility, the results were not robust to multiple testing due to limited statistical power. Although this is one of the largest UCCB studies carried out so far, it was not designed to have sufficient power to investigate heterogeneity by subphenotypes. However, the study provides valuable novel information on the heterogeneity in genetic susceptibility of UCCB that, jointly with the molecular and pathologic heterogeneity, will help to disentangle the complexity of this disease. This work should stimulate replication of our findings in independent UCCB case series.

5. Conclusions

To our knowledge, this is the first report to investigate heterogeneity of genetic susceptibility to subphenotypes defined by clinicopathologic characteristics in a large epidemiologic study. Importantly, the well-established variants associated with UCCB displayed similar risks for all subphenotypes. Furthermore, we identified new polymorphisms potentially associated with particular subphenotypes that did not show overall associations with UCCB risk. These exploratory analyses suggest that inherited factors may also contribute to the molecular/pathologic diversity of UCCB. However, validation through large-scale replication studies is required. Ongoing genome-wide association studies should contribute to these aims [31].

Although this research may not have immediate consequences at the clinical level, these results help to disentangle the molecular and pathologic diversity of UCCB. Furthermore, novel genetic associations with particular subphenotypes could lead to the discovery of novel genes involved in disease progression and to novel therapeutic targets. The relevance of tumour subphenotypes in cancer treatment is becoming obvious in areas where there is more knowledge, such as is the case for breast cancer (ie, luminal vs basal) or colorectal cancer (ie, mismatch repair vs nonmismatch repair). UCCB offers an opportunity in this sense.

Take-home message

This report is the first to examine genetic susceptibility to distinct bladder cancer subphenotypes. We provide evidence of heterogeneity in variant risk estimates among bladder subphenotypes. Replication will be required for conclusive evidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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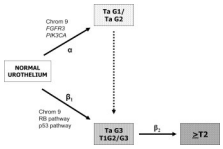


Fig. 1.

A model for bladder cancer development and progression. Two pathways are defined according to distinctive bladder cancer subphenotypes: The first pathway is composed of low-grade non–muscle-invasive (Ta G1/Ta G2)—genomic stable—tumours; the second is composed of both high-grade non–muscle-invasive (Ta G3/T1 G2/G3) and muscle-invasive (tumour stage [T] ≥2)—genomic unstable—tumours. Mutations in *FGFR3* and *PIK3CA* have been shown to be associated with the first pathway, whereas alterations in the p53 and RB functional networks have been shown to be important for the second pathway. α, β_1, and β_2 refer to the genetic variants associated with low-grade non–muscle-invasive, high-grade non–muscle-invasive, and muscle-invasive tumours, respectively.

G = histologic grade.

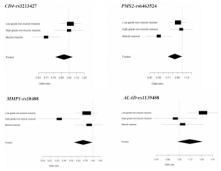
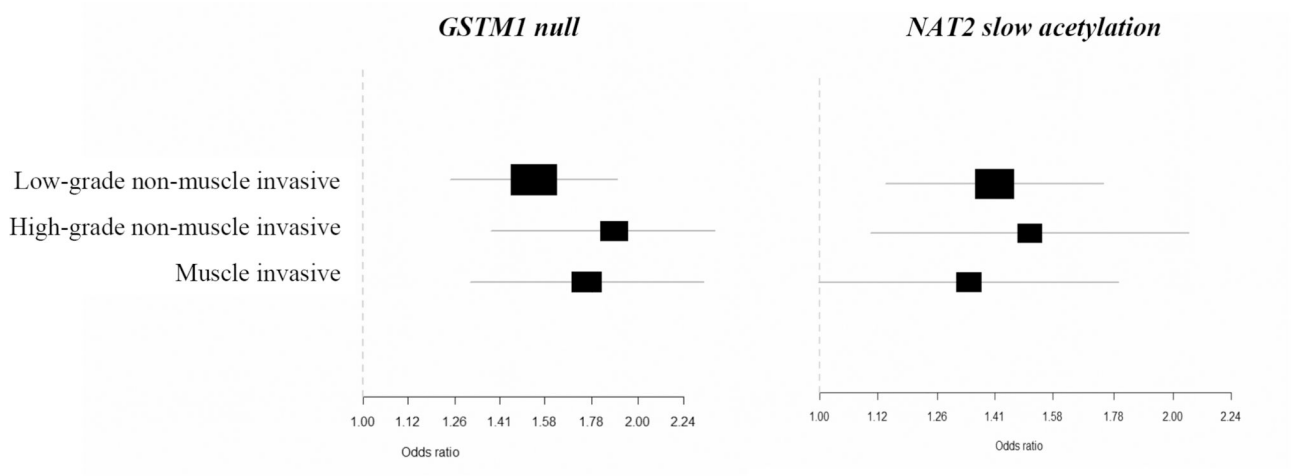


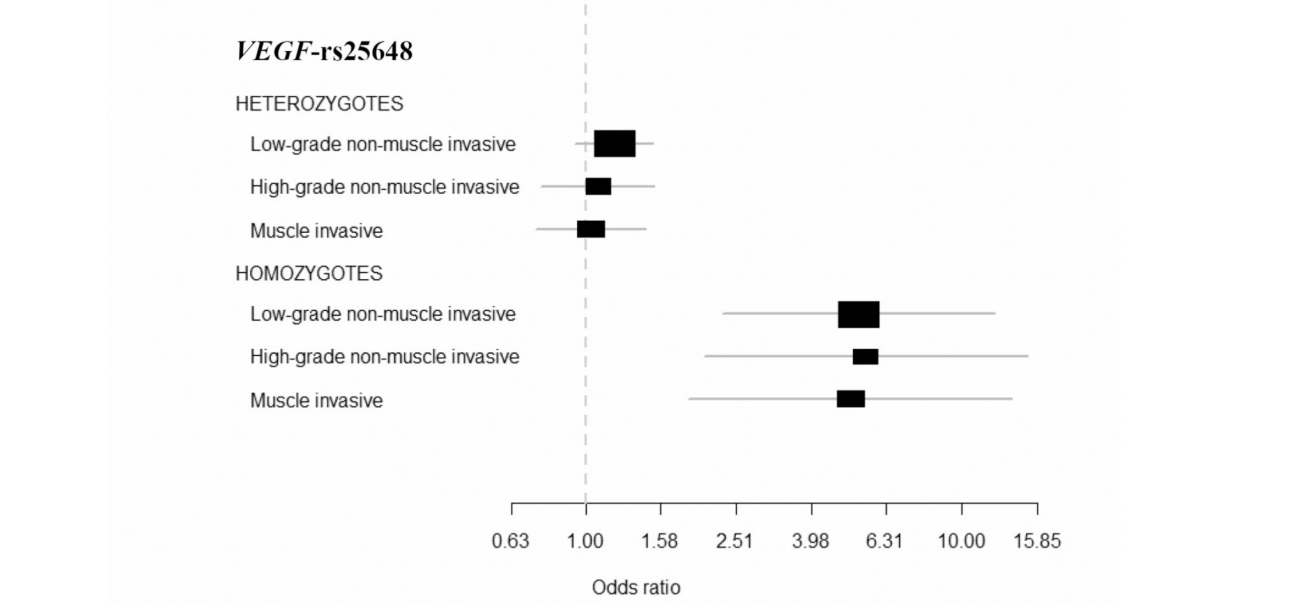
Fig. 2.

Per-allele risk estimates and 95% confidence intervals for bladder cancer subphenotypes and pooled cases. Per-allele risk estimates for bladder cancer subphenotypes are represented by boxes that are proportional to sample size.

LRT = likelihood ratio test; OR = odds ratio.



	1: Low-grade non-muscle invasive				2: High-grade non-muscle invasive				3: Muscle-invasive				LRT *P*-values	
Gene	*OR*	*L95*	*U95*	*p*	*OR*	*L95*	*U95*	*p*	*OR*	*L95*	*U95*	*p*	*Global*	*Hetero*
GSTM1	1.537	1.247	1.896	0.0001	1.881	1.379	2.564	0.0001	1.756	1.308	2.358	0.0002	0.000001	0.422
NAT2	1.409	1.138	1.745	0.002	1.509	1.106	2.060	0.009	1.339	0.998	1.797	0.050	0.003	0.830



	1: Low-grade non-muscle invasive				2: High-grade non-muscle invasive				3: Muscle-invasive				LRT *P*-values	
variant	*OR*	*L95*	*U95*	*LRT p*	*OR*	*L95*	*U95*	*LRT p*	*OR*	*L95*	*U95*	*LRT p*	*Global*	*Hetero*
het	1.189	0.935	1.512	0.0001	1.072	0.755	1.521	0.004	1.027	0.733	1.440	0.008	0.0005	0.936
hom	5.297	2.299	12.207		5.551	2.059	14.963		5.059	1.877	13.631			

Fig. 3.

(a) Risk estimates and 95% confidence intervals (CIs) for bladder cancer subphenotypes for *GSTM1*-null genotype and *NAT2* slow acetylation. (b) Risk estimates and 95% CIs for bladder cancer subphenotypes for the *VEGF*-rs25648 single nucleotide polymorphism (SNP), given a codominant mode of inheritance. Risk estimates for bladder cancer subphenotypes are represented by boxes that are proportional to sample size. The "Global" *p* value tests for a genetic association with any subphenotype. The "Hetero" *p* value tests for heterogeneity in genotype risk estimates among all subphenotypes.

Table 1

Characteristics of the Spanish Bladder Cancer/EPICURO study population*

	Bladder cancer subphenotypes			Controls	Group comparisons			
	Non–muscle invasive		Muscle invasive					
	Low grade	High grade			All groups		Case groups only	
	(*n* = 586)	(*n* = 219)	(*n* = 246)	(*n* = 1149)	χ^{2a}	*p* value	χ^{2a}	*p* value
Stage/grade**								
Ta G1	309 (52.7)	–	–	–	–	–	–	–
Ta G2	277 (47.3)	–	–	–	–	–	–	–
Ta G3	–	84 (38.4)	–	–	–	–	–	–
T1 G2	–	21 (9.6)	–	–	–	–	–	–
T1 G3	–	114 (52.1)	–	–	–	–	–	–
T2 G2	–	–	10 (4.1)	–	–	–	–	–
T2 G3	–	–	124 (50.4)	–	–	–	–	–
T3 G2	–	–	6 (2.4)	–	–	–	–	–
T3 G3	–	–	53 (21.5)	–	–	–	–	–
T4 G2	–	–	6 (2.4)	–	–	–	–	–
T4 G3	–	–	47 (19.1)	–	–	–	–	–
Age, mean (SD)	65.2 (10.7)	67.3 (8.5)	67.1 (9.1)	64.7 (9.9)	$F_{3,643.2} = 8.5$	0.00002	$F_{2,520.3} = 5.5$	0.005
Male gender	514 (87.7)	191 (87.2)	217 (88.2)	1002 (87.2)	0.2	0.9	0.1	0.9
Region								
Barcelona	107 (18.3)	45 (20.5)	39 (15.9)	232 (20.2)	7.8	0.8	5.7	0.7
Vallès/Bages	92 (15.7)	43 (19.6)	44 (17.9)	182 (15.8)	–	–	–	–
Alicante	42 (7.2)	13 (5.9)	17 (6.9)	82 (7.1)	–	–	–	–
Tenerife	99 (16.9)	39 (17.8)	49 (19.9)	191 (16.6)	–	–	–	–
Asturias	246 (42.0)	79 (36.1)	97 (39.4)	462 (40.2)	–	–	–	–
Smoking status*b*								
Never	76 (13.0)	29 (13.2)	33 (13.4)	338 (29.4)	128.8	<0.00001	4.2	0.7
Occasional	27 (4.6)	9 (4.1)	13 (5.3)	88 (7.7)	–	–	–	–

	Bladder cancer subphenotypes			Controls	Group comparisons			
	Non–muscle invasive		Muscle invasive		All groups		Case groups only	
	Low grade (*n* = 586)	High grade (*n* = 219)	(*n* = 246)	(*n* = 1149)	χ^{2a}	*p* value	χ^{2a}	*p* value
Former	235 (40.1)	93 (42.5)	84 (34.1)	428 (37.2)	–	–	–	–
Current	248 (42.3)	88 (40.2)	116 (47.2)	295 (25.7)	–	–	–	–

T = tumour stage; G = histologic grade; rs = reference single nucleotide polymorphism (SNP) number; SD = standard deviation.

*Data are presented as number of participants and column percentages unless otherwise specified.

**Fifty-eight cases could not be assigned to any T-G group because the paraffin block could not be retrieved.

[a]χ^2 test of independence for categorical variables and one-way analysis of variance for continuous variables.

[b]Specification of smoking status variable: *never*, those who smoked <100 cigarettes in their lifetime; *occasional*, those who smoked <1 cigarette per day for 6 mo; *former*, those who smoked at least 1 cigarette per day for 6 mo but did not smoke within 1 yr previous to the interview date; *current*, those who smoked at least 1 cigarette per day within 1 yr of the interview date.

Table 2

Heterogeneity in single nucleotide polymorphism (SNP)* risk estimates among bladder cancer subphenotypes in the Spanish Bladder Cancer Study ($p \leq 0.01$)

(a) Codominant mode of inheritance

			1: Low-grade non–muscle invasive				2: High-grade non–muscle invasive				3: Muscle invasive				LRT *p* values				
Gene	**rs no.**	**Variant[a]**	**OR**	**L95**	**U95**	**LRT *p***	**OR**	**L95**	**U95**	**LRT *p***	**OR**	**L95**	**U95**	**LRT *p***	**Global[b]**	**Hetero[c]**	**1 = 2[d]**	**1 = 3[d]**	**2 = 3[d]**
PMS2	rs6463524	*het*	0.945	0.747	1.196	0.443	1.181	0.853	1.637	0.057	0.512	0.355	0.738	0.001	0.001	0.002	0.179	0.005	0.000
		hom	0.666	0.344	1.292		0.281	0.066	1.192		0.543	0.207	1.425						
IGF1	rs5742665	*het*	0.957	0.751	1.220	0.538	0.676	0.462	0.990	0.084	1.407	1.026	1.929	0.019	0.007	0.002	0.219	0.004	0.000
		hom	1.363	0.750	2.477		1.305	0.571	2.983		0.342	0.080	1.468						
DNAJC1 8	rs4315920	*het*	0.879	0.701	1.103	0.312	0.831	0.601	1.149	0.229	1.182	0.855	1.635	0.033	0.016	0.005	0.809	0.003	0.004
		hom	0.785	0.549	1.123		0.653	0.377	1.129		1.798	1.167	2.769						
XRCC5	rs828702	*het*	1.018	0.793	1.308	0.733	1.552	1.056	2.282	0.039	0.747	0.538	1.038	0.099	0.023	0.006	0.098	0.050	0.001
		hom	1.118	0.830	1.505		1.653	1.058	2.584		0.663	0.435	1.009						
MBL2	rs5030737	*het*	1.016	0.758	1.361	0.836	0.457	0.265	0.788	0.006	1.005	0.671	1.507	0.979	0.021	0.007	0.004	0.528	0.005
		hom	0.601	0.105	3.431		2.088	0.436	9.986		–	–	–						
ABCC4	rs2274406	*het*	1.039	0.818	1.319	0.747	0.765	0.541	1.082	0.169	1.042	0.757	1.433	0.013	0.023	0.009	0.079	0.066	0.002
		hom	0.921	0.667	1.272		1.090	0.711	1.672		0.522	0.313	0.871						
BRCA2	rs1801406	*het*	1.141	0.913	1.424	0.475	1.113	0.801	1.546	0.010	0.851	0.626	1.157	0.392	0.021	0.009	0.040	0.112	0.005
		hom	1.147	0.760	1.731		2.202	1.341	3.617		0.708	0.376	1.331						
MMP1	rs10488	*het*	0.862	0.606	1.226	0.349	0.275	0.125	0.603	0.001	0.966	0.606	1.540	0.885	0.007	0.010	0.005	0.660	0.004
		hom	0.305	0.035	2.643		0.816	0.093	7.182		–	–	–						

(b) Additive mode of inheritance

			1: Low-grade non–muscle invasive				2: High-grade non–muscle invasive				3: Muscle invasive				LRT *p* values				
Gene	**rs no.**		**OR**	**L95**	**U95**	***p***	**OR**	**L95**	**U95**	***p***	**OR**	**L95**	**U95**	***p***	**Global[b]**	**Hetero[c]**	**1 = 2[d]**	**1 = 3[d]**	**2 = 3[d]**
DNAJC1 8	rs 4315920	Per allele	0.884	0.754	1.037	0.128	0.818	0.648	1.033	0.089	1.309	1.061	1.616	0.013	0.002	0.001	0.535	0.001	0.001
XRCC5	rs 828702	Per allele	1.056	0.909	1.225	0.477	1.276	1.030	1.582	0.026	0.802	0.651	0.988	0.037	0.006	0.002	0.098	0.014	0.001
SLC23A1	rs 10063949	Per allele	0.889	0.760	1.039	0.139	0.816	0.649	1.027	0.080	1.249	1.012	1.541	0.039	0.007	0.003	0.489	0.003	0.003
CD4	rs 3213427	Per allele	1.020	0.875	1.190	0.797	1.000	0.801	1.248	0.999	0.706	0.570	0.876	0.001	0.008	0.004	0.864	0.001	0.013
SLC23A1	rs 6596471	Per allele	0.882	0.751	1.037	0.128	0.987	0.785	1.240	0.909	1.292	1.045	1.598	0.019	0.013	0.005	0.368	0.001	0.056

(b) Additive mode of inheritance

Gene	rs no.		1: Low-grade non–muscle invasive				2: High-grade non–muscle invasive				3: Muscle invasive				LRT *p* values				
			OR	L95	U95	*p*	OR	L95	U95	*p*	OR	L95	U95	*p*	Global[b]	Hetero[c]	1 = 2[d]	1 = 3[d]	2 = 3[d]
PMS2	rs 6463524	Per allele	0.900	0.737	1.099	0.300	0.975	0.735	1.293	0.861	0.561	0.408	0.770	0.0002	0.002	0.006	0.601	0.004	0.005
BRCA2	rs 1801406	Per allele	1.100	0.931	1.300	0.264	1.361	1.078	1.718	0.010	0.848	0.666	1.078	0.174	0.011	0.007	0.090	0.040	0.002
CFH	rs 2274700	Per allele	0.902	0.774	1.052	0.190	1.162	0.935	1.443	0.177	1.235	1.004	1.520	0.046	0.019	0.007	0.031	0.005	0.651
MATR3	rs 11738738	Per allele	0.884	0.754	1.036	0.127	0.963	0.768	1.208	0.745	1.262	1.021	1.559	0.032	0.022	0.008	0.483	0.002	0.055

LRT = likelihood ratio test; OR = odds ratio; rs = reference number for SNPs.

[*] See Supplementary Table 1 for details about these SNPs. SNPs are ordered according to the most significant heterogeneity *p* value.

[a] The common homozygote genotype is the reference category in all models. ORs are adjusted for age, gender, region, and smoking status.

[b] The “Global” LRT (6 df, codominant model; 3 df, additive model) tests for a genetic association with any bladder cancer subphenotype.

[c] The “Hetero” LRT (4 df, codominant model; 2 df, additive model) tests for heterogeneity in risk estimates among all subphenotypes.

[d] Post hoc pairwise comparisons (LRT: 2 df, codominant model; 1 df, additive model) performed for those SNPs that exhibited heterogeneity in risk estimates (ie, “Hetero” $p \leq 0.01$): 1 = 2, compares risk estimates between the non–muscle-invasive groups; 1 = 3, compares risk estimates between the low-grade non–muscle-invasive and muscle-invasive groups; 2 = 3, compares risk estimates between the high-grade non–muscle-invasive and muscle-invasive groups.

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Table 3

Risk estimates for pooled tumour subphenotypes for those single nucleotide polymorphisms (SNPs)* that exhibited genetic heterogeneity

		Heterozygous[a]			Homozygous variant[a]				Per-allele risk			
Gene	**rs no.**	**OR**	**L95**	**U95**	**OR**	**L95**	**U95**	**LRT *p***	**OR**	**L95**	**U95**	***p***
ABCC4	rs2274406	0.980	0.799	1.203	0.863	0.654	1.139	0.560	0.939	0.822	1.073	0.353
BRCA2	rs1801406	1.060	0.875	1.284	1.229	0.868	1.740	0.482	1.087	0.942	1.255	0.253
CD4	rs3213427	1.079	0.865	1.346	0.865	0.664	1.127	0.171	0.934	0.819	1.066	0.313
DNAJC18	rs4315920	0.924	0.760	1.123	0.942	0.699	1.268	0.721	0.956	0.835	1.094	0.511
CFH	rs2274700	1.080	0.884	1.321	1.019	0.776	1.338	0.740	1.023	0.898	1.166	0.731
IGF1	rs5742665	0.987	0.803	1.215	1.138	0.660	1.961	0.885	1.015	0.853	1.206	0.869
MATR3	rs11738738	1.008	0.828	1.227	0.933	0.696	1.249	0.865	0.977	0.854	1.119	0.741
MBL2	rs5030737	0.890	0.688	1.151	0.821	0.209	3.226	0.651	0.892	0.700	1.137	0.355
MMP1	rs10488	0.758	0.556	1.032	0.347	0.066	1.821	0.093	0.734	0.550	0.980	0.036
PMS2	rs6463524	0.879	0.717	1.077	0.556	0.313	0.990	0.075	0.834	0.702	0.991	0.039
SLC23A1	rs10063949	0.947	0.777	1.154	0.894	0.672	1.190	0.716	0.946	0.828	1.081	0.414
SLC23A1	rs6596471	1.064	0.876	1.292	0.908	0.669	1.233	0.576	0.988	0.863	1.133	0.867
XRCC5	rs828702	1.018	0.822	1.261	1.065	0.823	1.377	0.888	1.031	0.907	1.172	0.641

LRT = likelihood ratio test; OR = odds ratio; rs = reference number for SNPs.

[a]The common homozygote genotype is the reference category in all models. ORs are adjusted for age, gender, region, and smoking status.

*See Supplementary Table 1 for details about these SNPs.